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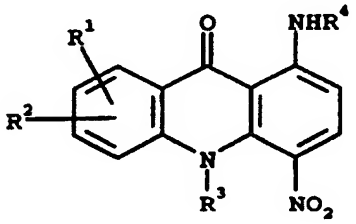
With international search report.

(54) Title: INHIBITION OF AMYLOIDOSIS BY 9-ACRIDINONES

(57) Abstract

Amyloid aggregation in animals is inhibited by administering a 9-acridinone compound of formula (I), wherein R^1 and R^2 are hydrogen, halo, nitro, amino, hydroxy, trifluoromethyl, alkyl, alkoxy, and alkylthio; R^3 is hydrogen or alkyl; and R^4 is -alkylene-NR^5R^6. The compounds are especially useful in preventing and treating Alzheimer's disease.



(I)

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INHIBITION OF AMYLOIDOSIS BY 9-ACRIDINONES

FIELD OF THE INVENTION

This invention concerns a method for inhibiting amyloidosis utilizing 9-acridinone compounds. The invention is a method for diagnosing and treating diseases characterized by amyloidosis.

BACKGROUND OF THE INVENTION

Amyloid plaque formation is found in a number of diseases, including Alzheimer's disease, scrapie, bovine spongiform encephalophy, Gerstmann-Straussler Syndrome, and the like. The amyloid plaques comprise proteins bound together in a fibrillous matrix. Amyloidosis is the general name given to diseases and conditions characterized by the presence of amyloid protein. A number of different types of amyloid protein are known, and all types are considered pathological, since no normally occurring amyloids are known. Accordingly, the presence of amyloid protein in a host is an indication of abnormal formation of fibrils and plaques. Amyloidosis has been clinically observed in a number of disease states, including certain mental illnesses, neurological diseases, and collagenosis. Indeed, the brains of subjects diagnosed with Alzheimer's disease have one thing in common, namely an abundance of amyloid in the form of plaques and tangles.

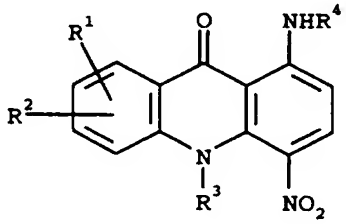
Alzheimer's disease is a degenerative brain disorder characterized clinically by progressive loss of memory, cognition, reasoning, judgement, and emotional stability that gradually leads to mental

deterioration and ultimately death. To date, only one clinically approved treatment is available, namely tacrine hydrochloride (Cognex®, from the Parke-Davis Division of Warner-Lambert Company). Because Alzheimer's disease and related degenerative brain disorders are a major medical issue for an aging population, the need for new treatments and methods for diagnosing the disorders are needed.

We have now discovered that certain 9-acridinone compounds inhibit amyloid aggregation. The acridinone compounds are described as antibacterial and antitumor agents by Capps in U.S. Patent No. 4,626,540. The compounds are also described as antitumor agents by Cholody, et al., in J. Med. Chem., 1990;33:49-52 and 1992;35:378-382. These references are incorporated herein by reference for their teaching of synthesis.

SUMMARY OF THE INVENTION

This invention provides a method for inhibiting amyloid aggregation in a mammal by administering a 9-acridinone compound. More particularly, the invention is a method for preventing amyloidosis comprising administering to a mammal an effective amount of a compound having the formula



wherein:

R^1 and R^2 independently are hydrogen, halo, nitro, amino, hydroxy, trifluoromethyl, C_1-C_4 alkyl-(O or S)$_{0 \text{ or } 1}$, or R^5R^6N-alkylene-(O or S)$_{0 \text{ or } 1}$;

R^3 is hydrogen or C_1-C_4 alkyl;

R^4 is -alkylene-NR^5R^6;

alkylene is a C_2-C_4 straight or branched hydrocarbon chain;

R^5 and R^6 independently are hydrogen, C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, or taken together with the nitrogen to which they are attached are piperidyl or pyrrolidinyl, and the pharmaceutically acceptable salts thereof.

A preferred method for inhibiting amyloid aggregation employs a compound of the above formula wherein R^1 and R^2 independently are hydrogen, hydroxy, C_1-C_4 alkyl-O-, or C_1-C_4 alkyl-S-.

Another preferred embodiment employs a compound of the above formula wherein R^4 is $-(CH_2)_n-NR^5R^6$;

R^5 and R^6 both are methyl or ethyl; and

n is 2 or 3.

The most preferred method of the invention employs compounds of the above formula wherein R^3 is hydrogen.

DETAILED DESCRIPTION OF THE INVENTION

In the above formula, R^1 and R^2 can be "C_1-C_4 alkyl (O or S)$_{0 \text{ or } 1}$". This term means a straight or branched alkyl group of up to 4 carbons, optionally bonded through oxygen or sulfur. Typical groups include methyl, methoxy, methylthio, ethoxy, ethylthio, isopropyl, isopropoxy, tert.-butoxy, and the like. R^1 and R^2 can additionally be "R^5R^6N-alkylene-(O or S)$_{0 \text{ or } 1}$." This term means a C_2-C_4 straight or branched alkylene group having attached to it an amino,

substituted amino, or disubstituted amino group, and optionally bonded through an oxygen or sulfur atom. Examples include 2-aminoethyl, 3-aminopropoxy, 2-amino-1-methylpropylthio, 2-methylaminoethyl, 2-N,N-diethylaminoethoxy, 3-piperidinopropyl, 4-pyrrolidinobutylthio, and the like.

R^4 is a C_2-C_4 alkylene group having attached to it a terminal amino, substituted or disubstituted amino group (NR^5R^6). The amino substituents can be C_1-C_4 alkyl or a C_1-C_4 alkyl having a hydroxy group attached as a substituent. Typical hydroxy -C_1-C_4 alkyl groups include hydroxymethyl, 3-hydroxypropyl, 4-hydroxybutyl, and the like. Examples of R^4 alkylene-NR^5R^6 groups therefore are 2-aminoethyl, 3-aminopropyl, 3-N-ethylaminopropyl, 3-(N-ethyl-N-hydroxymethyl)propyl, 3-pyrrolidinopropyl, and the like.

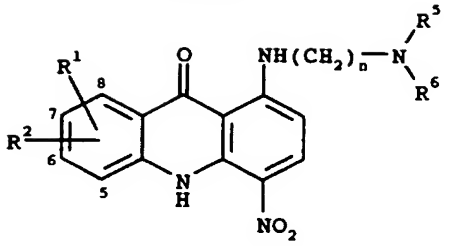
The compounds to be utilized in the method of this invention are known. The compounds preferably are employed as acid addition salts, thereby facilitating oral absorption and solubility. The pharmaceutically acceptable salts are prepared in normal fashion by reacting an amine of the above formula with an organic or inorganic acid such as citric acid, oxalic acid, hydrochloric acid, and the like.

The ability of the 9-acridinone compounds of the above formula to inhibit amyloid aggregation has been established in a standard in vitro assay. The assay is carried out by mixing beta amyloid peptide (1-40) with radioiodinated (I^{125}) labeled peptide to a concentration of 2.5 mg/mL in hexafluoroisopropanol. The mixture is diluted 1 to 5 with water (v/v). Ten milliliters of the solution is mixed with 25 µL of 25 mM sodium phosphate buffer pH 6.0. The mixture is allowed to aggregate for 2 hours at room temperature with and without a test compound present. The mixtures are then diluted to 235 µL with phosphate buffer to

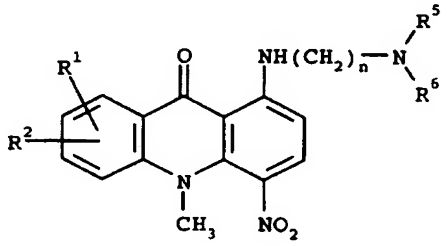
stop the aggregation process. The solutions are passed through a 0.2-μm millipore filtermat. Aggregated protein remains in the filter well. The filter plate is washed with 50 μL of phosphate buffer and then soaked in solid gel scintillant and counted on a Microbeta counter to determine the amount of aggregation in the presence of a test compound versus control with no test compound.

Several representative 9-acridinone compounds have been evaluated in the above assay and shown to inhibit amyloid aggregation. The following table presents the activity of selected compounds, reported as the molar concentration of compounds required to cause a 50% inhibition (IC_{50}) of amyloid aggregation in the above assay.

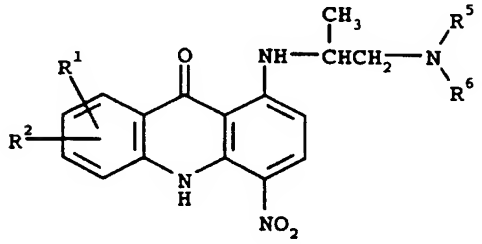
TABLE I



Compound No.	R^1	R^2	n	R^5	R^6	IC_{50} (μM)
1	7-SMe	H	3	Me	Me	7.1
2	7-SMe	H	2	Et	Et	9.0
3	6-OMe	7-OMe	2	Et	Et	11.0
4	7-OBu	H	2	Me	Me	25.0
5	7-OMe	H	3	Et	Et	21.0
6	7-OMe	6-Cl	2	Et	Et	18.7
7	7-O$(CH_2)_2NEt_2$	H	2	Et	Et	35.6
8	7-OEt	H	2	-$(CH_2)_2OH$	-$(CH_2)_2OH$	20.0
9	7-OH	H	2	Et	Et	20.5
10	7-OMe	H	2	Et	Et	13.9
11	H	H	2	Et	Et	29.5



12	7-OMe	H	2	Et	Et	23.2



13	7-OEt	H	4	Me	Me	24.3

The compounds of Formula I also have been evaluated utilizing human brain tissue. In a typical experiment, 30 µmol of compound (e.g., Compound No. 9) was mixed with 20 µmol of I^{125}-radiolabelled β-amyloid peptide (1-40) in a solution of 50 mmol of Tris (ph = 7.4) containing 4% (v/v) of bovine serum albumin to reduce nonspecific binding. The solution was stored at 25°C for 1 hour. Thin sections (about 20 µmeters) of human cadaver brain tissue were affixed to glass slides, and the slides were placed in the amyloid solution for 6 hours at 25°C. The glass slides were withdrawn, rinsed with cold (10°C) phosphate-buffered saline (PBS), fixed in 5% glutaraldehyde, and finally rinsed again sequentially with PBS and dehydrated ethanol. The slides were X-rayed using a Phosphorimager cassette (Molecular Dynamics) and dipped in photographic emulsion.

The brain tissues exposed to Compound No. 9 had 20% to 30% less radioactive grain accumulations when compared to untreated brain tissue. The grain accumulations are associated with amyloid plaques. The data thus demonstrates the test compound decreases the number and size of amyloid plaques. No grain accumulations appeared in human cerebellar sections, or assocated with blood vessels.

For inhibition of amyloid aggregation according to this invention, all that is required is to administer to a mammal an effective amount of a 9-acridinone compound as defined above. An "effective amount" as used herein is that quantity of 9-acridinone compound which inhibits aggregation of amyloid protein without causing unacceptable toxic effects. Typical doses which are effective will be from about 0.1 to about 1000 mg/day, and more typically from about 50 to about 500 mg/day. The compounds can be administered from one to about three times a day for either

prophylactic or therapeutic treatment of diseases related to the deposition of one or more amyloidogenic proteins, for example Alzheimer's disease, Down's syndrome, and in general advanced aging of the brain.

The 9-acridinone compounds can be formulated for convenient administration orally or parenterally, for instance by intravenous or intramuscular routes. The compounds also are well suited to transdermal delivery, and can thus be formulated as patches, creams, lotions, and the like. Typical formulations for oral administration will be made by mixing the 9-acridinone compound with common diluents and excipients such as corn starch, sugar, talc, and the like, and forming tablets, capsules, caplets, syrups, suspensions, and the like. For parenteral delivery, the compounds are ideally dissolved in isotonic saline or aqueous glucose for injection or intravenous delivery. The compounds can also be formulated with waxes and polymers and molded into suppositories or other common sustained-release delivery forms. The 9-acridinone compounds are preferably converted to pharmaceutically acceptable salts to increase solubility and facilitate formulation and administration.

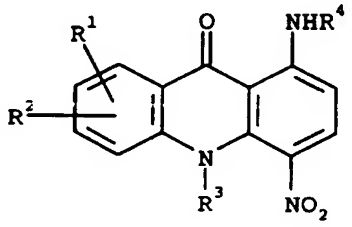
Because the 9-acridinone compounds described above are also effective at binding to amyloids, they can additionally be utilized to detect amyloid deposition, and thus to detect disease states associated with amyloid aggregation, such as Alzheimer's disease.

The compounds can readily be radiolabeled with common radioisotopes such as I^{125}, C^{11}, tritium, or the like. For example, compounds wherein R^1 or R^2 are halo can be made with I^{125}. Any of the carbons present in the compounds can be C^{11}. The radiolabeled compounds are synthesized as described in the references cited above, and employing common synthetic techniques

utilizing readily available radioactive chemicals. The radiolabeled compound is then formulated and administered to a mammal in the same manner as described above for nonradiolabeled compounds. The mammal can then be scanned with common imaging sensors and equipment to detect amyloid deposition and aggregation.

CLAIMS

1. A method for inhibiting amyloid aggregation in a mammal comprising administering an effective amount of a compound having the formula



wherein:

R^1 and R^2 independently are hydrogen, halo, nitro, amino, hydroxy, trifluoromethyl, C_1-C_4 alkyl-(O or S)$_{0 \text{ or } 1}$, or R^5R^6N-alkylene-(O or S)$_{0 \text{ or } 1}$;

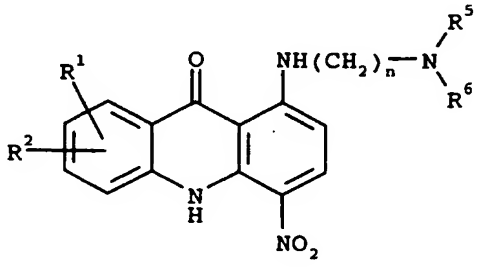
R^3 is hydrogen or C_1-C_4 alkyl;

R^4 is -alkylene-NR^5R^6;

alkylene is a C_2-C_4 straight or branched hydrocarbon chain;

R^5 and R^6 independently are hydrogen, C_1-C_4 alkyl, hydroxy-C_1-C_4 alkyl, or taken together with the nitrogen to which they are attached are piperidyl or pyrrolidinyl, and the pharmaceutically acceptable salts thereof;

2. A method according to Claim 1 employing a compound having the formula



wherein:

R^1 and R^2 independently are hydrogen, hydroxy, C_1-C_4 alkyl-O-, or C_1-C_4 alkyl-S-;

R^5 and R^6 both are methyl or ethyl;

and n is 2 or 3.

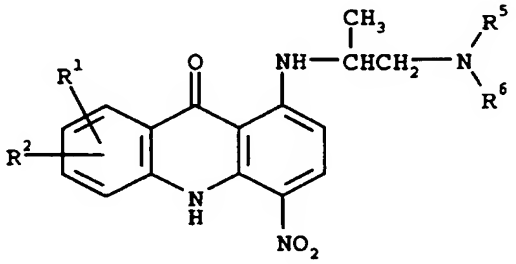
3. A method according to Claim 2 employing a compound wherein n is 2 and R^5 and R^6 both are ethyl.

4. A method according to Claim 3 employing a compound wherein R^1 is hydrogen, 7-methylthio, 7-methoxy or 7-hydroxy, and R^2 is hydrogen, 6-chloro, or 6-methoxy.

5. A method according to Claim 2 employing a compound wherein n is 3 and R^5 and R^6 both are methyl or ethyl.

6. A method according to Claim 5 employing a compound wherein R^1 is 7-methoxy or 7-methylthio, and R^2 is hydrogen.

7. A method according to Claim 1 employing a compound having the formula



wherein:

R^1 and R^2 independently are hydrogen, hydroxy, C_1-C_4 alkyl-O-, or C_1-C_4 alkyl-S-; and

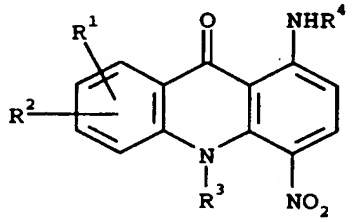
R^5 and R^6 both are methyl or ethyl.

8. A method according to Claim 7 employing a compound wherein R^1 is 7-ethoxy, and R^2 is hydrogen.

9. A method according to Claim 1 employing a compound wherein R^3 is C_1-C_4 alkyl.

10. A method according to Claim 9 employing a compound wherein R^3 is methyl.

11. A method of diagnosing a mammal having amyloid aggregation comprising administering an effective amount of a radiolabeled compound of the formula



wherein:

R^1 and R^2 independently are hydrogen, halo, nitro, amino, hydroxy, trifluoromethyl, C_1-C_4 alkyl-(O or S)$_{0 \text{ or } 1}$, or R^5R^6N-alkylene-(O or S)$_{0 \text{ or } 1}$;

R^3 is hydrogen or C_1-C_4 alkyl;

R^4 is -alkylene-NR^5R^6;

alkylene is a C_2-C_4 straight or branched hydrocarbon chain;

R^5 and R^6 independently are hydrogen, C_1-C_4 alkyl, or taken together with the nitrogen to which they are attached are piperidyl or pyrrolidinyl, and the pharmaceutically acceptable salts thereof;

and wherein at least 1 atom is radioactive, and imaging the mammal to determine the accumulation of the compound in brain tissue.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/16745

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/47 A61K51/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 145 226 A (WARNER-LAMBERT COMPANY) 19 June 1985 cited in the application see page 4 - page 5 & US 4 626 540 A (CAPPS)	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
30 January 1997	07.02.97

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir ·ational application No.
PCT/US 96/16745

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1-11 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Bux II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, t s International Search Report covers all searchable claims.

2 [ ] As all searchable claims could be searches without effort justifying an ad ·tional fee, this Authority did not invite payment of any additional fee.

3 [ ] As only some of the required additional search fees were timely paid by t. e applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

.nformation on patent family members

Inter nal Application No
PCT/US 96/16745

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0145226	19-06-85	US-A-	4626540	02-12-86
		AU-B-	573639	16-06-88
		AU-A-	3513184	16-05-85
		CA-A-	1258856	29-08-89
		DE-A-	3474446	10-11-88
		JP-A-	60136567	20-07-85